IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : Confirmation No. 7495
YOKOYAMA, et al. : Docket No. S-2481/CONT
Serial No. 10/666,384 : Group Art Unit 1618
Filed: September 22, 2003 : Examiner: James W. Rogers

PRODUCTION PROCESS FOR POLYMERIC MICELLE CHARGED THEREIN WITH DRUG AND POLYMERIC MICELLE COMPOSITION

DECLARATION UNDER RULE 1.132

Honorable Commissioner of
Patents and Trademarks
Washington, D.C.

Sir:

I, Chieko Tsuchiya, hereby declare as follows:

That I am a researcher of NanoCarrier Co., Ltd.

That I finished, in March 31, 2000, the Master Course of Chemical and Biological Science and Technology at the Department of Engineering of Graduate School of Tokyo University of Agriculture and Technology;.

That, in April 1 of the same year, I joined NanoCarrier Co., Ltd., where I have since engaged chiefly in the research and development of process for the production of medicine-encapsulated polymer micelle;

That I am therefore conversant with how to manufacture medicine-encapsulated polymer micelle;

That I am familiar with the above-identified patent application and with Ichiro et al. reference (JP 11-335267) which has been cited by the Examiner;

That the following experiments were carried out by myself, or under my supervision and control.

Experiments

The process as mentioned in Ichiro et al. for the production of medicine-encapsulated polymer micelle was conducted with use of a block copolymer as employed in Ichiro et al. corresponding to PEG-P (Asp, BLA), and with use of DMF and DMSO as solvents, and by using, as a medicine, paclitaxel which is assiduously studied in Examples of the present application. Method and results of the experiments were as follows.

1. Study of micelle formation with use of DMF:

Method:

Micelle formation was conducted by means of dialysis in accordance with the method as disclosed in JP 11-335267 A.

In detail, 100 mg of PEG-PBLA 12-40 (average molecular weight of polyethylene glycol: 12000; average polymerization degree of polybenzyl aspartate: 40; proportion of benzyl which remains after the hydrolysis of polybenzyl aspartate unit: about 60 %; proportion of hydrogen atom (i.e., proportion of polyaspartic acid which is formed as a result of hydrolysis): about 40 %) was dissolved in 1 mL of DMF, which operation was repeated three times to give three solutions. Separately, 5 mg, 10 mg and 30 mg of paclitaxel was dissolved in 1 mL of DMF to give three solutions. Thus prepared solutions of paclitaxel were respectively mixed with the above-prepared polymer solutions; the resultant mixtures were each stirred for 10 minutes at room temperature in a dark place. Each mixture (2 mL) was dialyzed against distilled water (1 L) through cellulose dialysis membrane (MWCO: 10,000) at room temperature. Two hours after the start of dialysis, distilled water (1 L) was replaced, and, then, dialysis was conducted for further 19 hours (i.e., total 21 hours).

Results:

The sample solutions dissolved in DMF began to show, from the sample which contained the largest amount of drug in the decreasing order, the formation of white precipitate (within two hours from the start of dialysis).

After 21 hours, white precipitate was still being formed. Although these samples were subjected to ultrasonication (Biodisruptor; 2 mL; 130 W; one-second intermittent irradiation for 10 minutes), white precipitate remained, and it was confirmed that no micelle had been formed.

2. Study of micelle formation with use of DMSO:

Method:

The method as mentioned in the above 1. was repeated except that DMSO was used as an organic solvent, in place of DMF.

Results:

The sample solutions dissolved in DMSO began to show the formation of white precipitate from immediately after the start of dialysis. After 21 hours, white precipitate was still being formed. Although these samples were subjected to ultrasonication (Biodisruptor; 2 mL; 130 W; one-second intermittent irradiation for 10 minutes), white precipitate remained, and it was confirmed that no micelle had been formed.

3. The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Signed this day of:

Aug. 30. 2006
Chieko Tsuchiya